MARKED UP VERSION OF AMENDMENTS

Specification Amendments Under 37 C.F.R. § 1.121(b)(1)(iii)

Replace the paragraph at page 2, lines 10 through 11 with the below paragraph marked up by way of bracketing and underlining to show the changes relative to the previous version of the paragraph.

One aspect of the invention is an isolated and purified polypeptide comprising the amino acid sequence of Figures 1A-1C (SEQ ID NO:2).

Replace the paragraph at page 2, lines 14 through 15 with the below paragraph marked up by way of bracketing and underlining to show the changes relative to the previous version of the paragraph.

One aspect of the invention is an isolated and purified polypeptide comprising the amino acid sequence of Figures 1A-1C [Figure 1] (SEQ ID NO:2).

Replace the paragraph at page 2, line 17 through 18, with the below paragraph marked up by way of bracketing and underlining to show the changes relative to the previous version of the paragraph.

Figures 1A-1C are [Figure 1 is] the DNA (SEQ ID NO:1) and deduced amino acid sequence (SEQ ID NO:2) of the human $P_{2U2}$ receptor.

Replace the paragraph at page 4, line 4 through 14 with the below paragraph marked up by way of bracketing and underlining to show the changes relative to the previous version of the paragraph.

The present invention relates to a new purinergic receptor of the $P_2$ subclass, which is referred to herein as the $P_{2U2}$ receptor. Figures 1A-1C show [Figure 1 shows] the DNA (SEQ ID NO:1) sequence of the clone encoding the $P_{2U2}$ receptor along with the deduced amino acid sequence. The amino acid sequence shown in Figures 1A-1C [Figure 1] (SEQ ID NO:1) include [includes] four putative extracellular domains (the $NH_2$ -terminus and ECD I-ECD III) and seven

putative transmembrane regions (TM I-TM VII). As used herein, the " $P_{2U2}$ receptor" refers to the receptor in any animal species sharing a common biological activity with the human receptor contained in the clone described in Example 1 herein. This "common biological activity" includes but is not limited to an effector or receptor function or cross-reactive antigenicity. Using the native DNA encoding the human form of this receptor, the $P_{2U2}$ receptors in other species, may be obtained.

Replace the paragraph at page 4, line 15 through 19 with the below paragraph marked up by way of bracketing and underlining to show the changes relative to the previous version of the paragraph.

Because the $P_{2U2}$ receptor is activated by UTP, it is classified as a $P_2$-type purinergic receptor. Hydrophobicity/hydrophilicity plots of the $P_{2U2}$ receptor sequence shown in Figures 1A-1C (SEQ ID NO:1) suggest that the $P_{2U2}$ receptor has 7 putative transmembrane domains. This, along with the following characteristics, are consistent with characteristics that are observed in other $P_2$-type purinergic receptors:

Replace the paragraphs at page 5, line 7 through 29 with the below paragraphs marked up by way of bracketing and underlining to show the changes relative to the previous version of the paragraphs.

One aspect of the present invention also relates to the human gene encoding the $P_{2U2}$ receptor, which has both diagnostic and therapeutic uses as are described below. Included within this invention are proteins or peptides having substantial homology with the amino acid sequence of Figures 1A-1C [Figure 1] (SEQ ID NO:1).

Ordinarily, the $P_{2U2}$ receptors and analogs thereof claimed herein will have an amino acid sequence having at least 75% amino acid sequence identity with the $P_{2U2}$ receptor sequence disclosed in Figures 1A-1C [Figure 1] (SEQ ID NO:1), more preferably at least 80%, even more preferably at least 90%, and most preferably at least 95%. Identity or homology with a sequence is defined herein as the percentage of amino acid residues in the candidate sequence that are identical with the sequence of the $P_{2U2}$ receptor, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent homology, and not considering any conservative substitutions as part of the sequence identity. None of N-terminal, C-terminal or internal extensions,

deletions, or insertions of the $P_{2U2}$ receptor sequence shall be construed as affecting homology.

Thus, the claimed $P_{2U2}$ receptor and analog molecules that are the subject of this invention include molecules having the $P_{2U2}$ receptor amino acid sequence; fragments thereof having a consecutive sequence of at least 10, 15, 20, 25, 30 or 40 amino acid residues from the $P_{2U2}$ receptor sequence of Figures 1A-1C [Figure 1] (SEQ ID NO:1), amino acid sequence variants of the $P_{2U2}$ receptor sequence of Figures 1A-1C [Figure 1] (SEQ IN NO:1) wherein an amino acid residue has been inserted N- or C-terminal to, or within, (including parallel deletions) the $P_{2U2}$ receptor sequence or its fragments as defined above; amino acid sequence variants of the $P_{2U2}$ receptor sequence of Figures 1A-1C [Figure 1] (SEQ ID NO:1) or its fragments as defined above which have been substituted by at least one residue.

Replace the paragraph at page 11, lines 13 through 18 with the below paragraph marked up by way of bracketing and underlining to show the changes relative to the previous version of the paragraph.

Recombinant production of the $P_{2U2}$ receptor involves using a nucleic acid sequence that encodes the $P_{2U2}$ receptor, as is set forth in Figures 1A-1C [Figure 1] (SEQ ID NO:1), or its degenerate analogs. The nucleic acid can be prepared either by retrieving the native sequence, as described below, or by using substantial portions of the known native sequence as a probe, or it can be synthesized de novo using procedures that are well known in the art.

Replace the paragraph at page 12, line 8 through page 13, line 6 with the below paragraph marked up by way of bracketing and underlining to show the changes relative to the previous version of the paragraph.

$P_{2U2}$ receptor nucleic acids for use in the invention can be produced as follows: A $P_{2U2}$ receptor "nucleic acid" is defined as RNA or DNA that encodes a $P_{2U2}$ receptor, or is complementary to nucleic acid sequence encoding a $P_{2U2}$ receptor, or hybridizes to such nucleic acid and remains stably bound to it under stringent conditions, or encodes a polypeptide sharing at least 75% sequence identity, preferably at least 80%, and more preferably at least 85%, with the translated amino acid sequence shown in Figures 1A-1C {Figure 1] (SEQ ID NO:1). It is typically at least about 10 nucleotides in length and preferably has $P_{2U2}$ receptor related biological or immunological activity.

Specifically contemplated are genomic DNA, cDNA, mRNA and antisense molecules, as well as nucleic acids based on alternative backbone or including alternative bases whether derived from natural sources or synthesized.

Replace the paragraph at page 17, line 3 through page 18, line 2 with the below paragraphs marked up by way of bracketing and underlining to show the changes relative to the previous version of the paragraphs.

In both the agonists and antagonists, a preferred embodiment is that class of compounds having amino acid sequences that are encoded by the $P_{2U2}$ receptor gene. Preferably, the agonists and antagonists have amino acid sequences, in whole or in part, corresponding to the extracellular domains of the $P_{2U2}$ receptor. For example, preferred peptides of the invention correspond, in whole or in part, to either the amino terminus, which is amino acid no 1, methionine (M) to amino acid no 23, lysine (K) (SEQ ID NO:5); ECD I, which is amino acid no 83, tyrosine (Y) to amino acid no 99, arginine (R) (SEQ ID NO:6); ECD II, which is amino acid no 162, asparagine (N) to amino acid no 183, tyrosine(Y) (SEQ ID NO:7); or ECD III, which is amino acid no 257, alanine (A) to amino acid no 276, phenylalanine (F) (SEQ ID NO:8). Also included in the invention are isolated DNA molecules that encode these specific peptides. Accordingly, the invention pertains to isolated DNA molecules encoding human $P_{2U2}$ receptor peptides comprising the amino acid sequence of Figures 1A-1C [Figure 1] from amino acid no 1, methionine to amino acid no 23, lysine (SEQ ID NO:5); from amino acid no 83, tyrosine to amino acid no 99, arginine (SEQ ID NO:6); from amino acid no 162, asparagine to amino acid no 183, tyrosine (SEQ ID NO:7); and from amino acid no 257, alanine to amino acid no 276, phenylalanine (SEQ ID NO:8).

The invention also includes agonists and antagonists that affect receptor function by binding to one of the intracellular (ICD) domains of the receptor. For example, preferred peptides within this aspect of the invention would correspond, in whole or in part, to either ICD I, which is amino acid no 50, phenylalanine (F) to amino acid no 60, isoleucine (I) (SEQ ID NO:11); ICD II, which is amino acid no 120, arginine (R) to amino acid no 141, leucine (L) (SEQ ID NO:12); ICD III, which is amino acid no 208, tyrosine (Y) to amino acid no 233, leucine (L) (SEQ ID NO:13); or to the carboxy terminus, which is amino acid no 301, histidine (H) to amino acid no 334, lysine (K) (SEQ ID NO:14). Also included in the invention are isolated DNA molecules that encode these specific

peptides. Accordingly, the invention pertains to isolated DNA molecules encoding human $P_{2U2}$ receptor peptides comprising the amino acid sequence of Figures 1A-1C [Figure 1] from amino acid no 50, phenylalanine to amino acid no 60, isoleucine (SEQ ID NO:11); amino acid no 120, arginine to amino acid no 141, leucine (SEQ ID NO:12); amino acid no 208, tyrosine to amino acid no 233, leucine (SEQ ID NO:13); and amino acid no 301, histidine (H) to amino acid no 334, lysine (K) (SEQ ID NO:14).

Replace the paragraph at page 25, line 24 through page 26, line 2 with the below paragraph marked up by way of bracketing and underlining to show the changes relative to the previous version of the paragraph.

To isolate additional 5' sequence for the $P_{2U2}$ gene, a 5' proximal fragment from the largest DAMI clone (D8) was used to screen a Clontech human kidney cDNA library ($\lambda$gt10) under identical screening conditions as were used for the DAMI cDNA library. DNA from plaque-purified positively hybridizing clones from both libraries were analyzed by restriction digest. Inserts from clones of interest were excised and subcloned into the commercially available pBluescript vector and sequenced as above. The complete open reading frame as well as truncated versions of the full-length cDNA were cloned into *Xenopus* oocyte or mammalian expression vectors for functional analysis. The DNA sequence of the complete open reading frame for the longest cDNA isolated from the kidney cDNA library is shown in Figures 1A-1C [Figure 1] (SEQ ID NO:1). As shown in Figure 2, the deduced amino acid sequence of the $P_{2U2}$ cDNA shows extensive homology with other known purinergic receptors (Parr, *supra*, and Henderson, supra).

sequence of Figures 1A-1C from amino acid no 50, phenylalanine to amino acid no 60, isoleucine (SEQ ID NO:11); amino acid no 120, arginine to amino acid no 141, leucine (SEQ ID NO:12); amino acid no 208, tyrosine to amino acid no 233, leucine (SEQ ID NO:13); and amino acid no 301, histidine (H) to amino acid no 334, lysine (K) (SEQ ID NO:14).

Please replace the paragraph at page 25, line 24 through page 26, line 2 with the following paragraph:

To isolate additional 5' sequence for the $P_{2U2}$ gene, a 5' proximal fragment from the largest DAMI clone (D8) was used to screen a Clontech human kidney cDNA library ($\lambda$gt10) under identical screening conditions as were used for the DAMI cDNA library. DNA from plaque-purified positively hybridizing clones from both libraries were analyzed by restriction digest. Inserts from clones of interest were excised and subcloned into the commercially available pBluescript vector and sequenced as above. The complete open reading frame as well as truncated versions of the full-length cDNA were cloned into *Xenopus* oocyte or mammalian expression vectors for functional analysis. The DNA sequence of the complete open reading frame for the longest cDNA isolated from the kidney cDNA library is shown in Figures 1A-1C (SEQ ID NO:1). As shown in Figure 2, the deduced amino acid sequence of the $P_{2U2}$ cDNA shows extensive homology with other known purinergic receptors (Parr, *supra*, and Henderson, supra).

Amendments to the specification are indicated in the attached "Marked Up Version of Amendments" (pages i-v).

## REMARKS

The subject application is a divisional of U.S. Patent Application No. 09/947,922 filed September 7, 2001.

In the Specification

Please amend the specification by inserting after the title the following:

Related Applications

This application is a divisional of U.S. Application Serial No. 09/947,922, filed September 7, 2001, which is a continuation of U.s. Application Serial No. 09/410,738, filed October 1, 1999 (abandoned), which is a continuation of U.S. Application Serial No. 08/749,707, filed November 15, 1996 (Patent No. 6,063,582), which is a continuation of U.S. Application No. 08/559,524, filed November 15, 1995 (Patent No. 5,871,963), which claims the benefit of U.S. Application Serial No. 60/006,782, filed November 15, 1995.

Please replace the paragraph at page 2, lines 10 through 11 with the following paragraph:

One aspect of the invention is an isolated and purified polypeptide comprising the amino acid sequence of Figures 1A-1C (SEQ ID NO:2).

Please replace the paragraph at page 2, lines 14 through 15 with the following paragraph:

Yet another aspect of the invention is an isolated and purified nucleic acid sequence comprising the nucleotide sequence of Figures 1A-1C (SEQ ID NO:2).

Please replace the paragraph at page 2, line 17 through 18, with the following paragraph:

Figures 1A-1C are the DNA (SEQ ID NO:1) and deduced amino acid sequence (SEQ ID NO:2) of the human $P_{2U2}$ receptor.

Please replace the paragraph at page 4, line 4 through 14 with the following paragraph:

The present invention relates to a new purinergic receptor of the $P_2$ subclass, which is referred to herein as the $P_{2U2}$ receptor. Figures 1A-1C show the DNA (SEQ ID NO:1) sequence of the clone encoding the $P_{2U2}$ receptor along with the deduced amino acid sequence. The amino acid sequence shown in Figures 1A-1C (SEQ ID NO:1) includes four putative extracellular domains (the $NH_2$ -terminus and ECD I-ECD III) and seven putative transmembrane regions (TM I-TM VII). As used herein, the " $P_{2U2}$ receptor" refers to the receptor in any animal species sharing a common biological activity with the human receptor contained in the clone described in

Example 1 herein. This "common biological activity" includes but is not limited to an effector or receptor function or cross-reactive antigenicity. Using the native DNA encoding the human form of this receptor, the $P_{2U2}$ receptors in other species, may be obtained.

Please replace the paragraph at page 4, line 15 through 19 with the following paragraph:

Because the $P_{2U2}$ receptor is activated by UTP, it is classified as a $P_2$-type purinergic receptor. Hydrophobicity/hydrophilicity plots of the $P_{2U2}$ receptor sequence shown in Figures 1A-1C (SEQ ID NO:1) suggest that the $P_{2U2}$ receptor has 7 putative transmembrane domains. This, along with the following characteristics, are consistent with characteristics that are observed in other $P_2$-type purinergic receptors:

Please replace the paragraphs at page 5, line 7 through 29 with the following paragraphs:

One aspect of the present invention also relates to the human gene encoding the $P_{2U2}$ receptor, which has both diagnostic and therapeutic uses as are described below. Included within this invention are proteins or peptides having substantial homology with the amino acid sequence of Figures 1A-1C (SEQ ID NO:1).

Ordinarily, the $P_{2U2}$ receptors and analogs thereof claimed herein will have an amino acid sequence having at least 75% amino acid sequence identity with the $P_{2U2}$ receptor sequence disclosed in Figures 1A-1C (SEQ ID NO:1), more preferably at least 80%, even more preferably at least 90%, and most preferably at least 95%. Identity or homology with a sequence is defined herein as the percentage of amino acid residues in the candidate sequence that are identical with the sequence of the $P_{2U2}$ receptor, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent homology, and not considering any conservative substitutions as part of the sequence identity. None of N-terminal, C-terminal or internal extensions, deletions, or insertions of the $P_{2U2}$ receptor sequence shall be construed as affecting homology.

Thus, the claimed $P_{2U2}$ receptor and analog molecules that are the subject of this invention include molecules having the $P_{2U2}$ receptor amino acid sequence; fragments thereof having a consecutive sequence of at least 10, 15, 20, 25, 30 or 40 amino acid residues from the $P_{2U2}$ receptor sequence of Figures 1A-1C (SEQ ID NO:1), amino acid sequence variants of the $P_{2U2}$ receptor sequence of Figures 1A-1C (SEQ IN NO:1) wherein an amino acid residue has

been inserted N- or C-terminal to, or within, (including parallel deletions) the $P_{2U2}$ receptor sequence or its fragments as defined above; amino acid sequence variants of the $P_{2U2}$ receptor sequence of Figures 1A-1C (SEQ ID NO:1) or its fragments as defined above which have been substituted by at least one residue.

Please replace the paragraph at page 11, lines 13 through 18 with the following paragraph:

Recombinant production of the $P_{2U2}$ receptor involves using a nucleic acid sequence that encodes the $P_{2U2}$ receptor, as is set forth in Figures 1A-1C (SEQ ID NO:1), or its degenerate analogs. The nucleic acid can be prepared either by retrieving the native sequence, as described below, or by using substantial portions of the known native sequence as a probe, or it can be synthesized de novo using procedures that are well known in the art.

Please replace the paragraph at page 12, line 28 through page 13, line 6 with the following paragraph:

$P_{2U2}$ receptor nucleic acids for use in the invention can be produced as follows: A $P_{2U2}$ receptor "nucleic acid" is defined as RNA or DNA that encodes a $P_{2U2}$ receptor, or is complementary to nucleic acid sequence encoding a $P_{2U2}$ receptor, or hybridizes to such nucleic acid and remains stably bound to it under stringent conditions, or encodes a polypeptide sharing at least 75% sequence identity, preferably at least 80%, and more preferably at least 85%, with the translated amino acid sequence shown in Figures 1A-1C (SEQ ID NO:1). It is typically at least about 10 nucleotides in length and preferably has $P_{2U2}$ receptor related biological or immunological activity. Specifically contemplated are genomic DNA, cDNA, mRNA and antisense molecules, as well as nucleic acids based on alternative backbone or including alternative bases whether derived from natural sources or synthesized.

Please replace the paragraph at page 17, line 3 through page 18, line 2 with the following paragraphs:

In both the agonists and antagonists, a preferred embodiment is that class of compounds having amino acid sequences that are encoded by the $P_{2U2}$ receptor gene. Preferably, the agonists and antagonists have amino acid sequences, in whole or in part, corresponding to the extracellular domains of the $P_{2U2}$ receptor. For example, preferred peptides of the invention correspond, in whole or in part, to either the amino terminus, which is amino acid no 1, methionine (M) to amino acid no 23, lysine (K) (SEQ ID NO:5); ECD I, which is amino acid no 83, tyrosine (Y) to amino acid no 99, arginine (R) (SEQ ID NO:6); ECD II, which is amino acid no 162, asparagine (N) to amino acid no 183, tyrosine(Y) (SEQ ID NO:7); or ECD III, which is amino acid no 257, alanine (A) to amino acid no 276, phenylalanine (F) (SEQ ID NO:8). Also included in the invention are isolated DNA molecules that encode these specific peptides. Accordingly, the invention pertains to isolated DNA molecules encoding human $P_{2U2}$ receptor peptides comprising the amino acid sequence of Figures 1A-1C from amino acid no 1, methionine to amino acid no 23, lysine (SEQ ID NO:5); from amino acid no 83, tyrosine to amino acid no 99, arginine (SEQ ID NO:6); from amino acid no 162, asparagine to amino acid no 183, tyrosine (SEQ ID NO:7); and from amino acid no 257, alanine to amino acid no 276, phenylalanine (SEQ ID NO:8).

The invention also includes agonists and antagonists that affect receptor function by binding to one of the intracellular (ICD) domains of the receptor. For example, preferred peptides within this aspect of the invention would correspond, in whole or in part, to either ICD I, which is amino acid no 50, phenylalanine (F) to amino acid no 60, isoleucine (I) (SEQ ID NO:11); ICD II, which is amino acid no 120, arginine (R) to amino acid no 141, leucine (L) (SEQ ID NO:12); ICD III, which is amino acid no 208, tyrosine (Y) to amino acid no 233, leucine (L) (SEQ ID NO:13); or to the carboxy terminus, which is amino acid no 301, histidine (H) to amino acid no 334, lysine (K) (SEQ ID NO:14). Also included in the invention are isolated DNA molecules that encode these specific peptides. Accordingly, the invention pertains to isolated DNA molecules encoding human $P_{2U2}$ receptor peptides comprising the amino acid

Amendments to the Specification

The Related Applications section of the Specification has been amended to recite that this application "is a divisional of U.S. Serial No. 09/947,922, filed September 7, 2001." In addition, the specification has been amended to refer to Figures 1A-1C so as to agree with the labeling of the Formal Drawings filed with the application on November 12, 2003.

This Amendment adds no new matter.

Respectfully submitted,
MILLENNIUM PHARMACEUTICALS, INC.

By _____

Jean M. Silveri
Registration No. 39,030
40 Landsdowne Street
Cambridge, MA 02139
Telephone: (617) 679-7336
Facsimile: (617) 551-8820

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